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	If the applicant is a corporate body, give the country/state of its incorporation	UNITED KINGDOM 7736630001
4.	Title of the invention	TREATMENT OF INFLAMMATORY BOWEL DISEASE
5.	Name of your agent *(if you have one)*	Marks & Clerk
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DUPLICATE

TREATMENT OF INFLAMMATORY BOWEL DISEASE

The present invention relates to the treatment of inflammatory bowel disease (IBD) and in particular treatment of IBD with soluble extracts derived from edible plants.

IBD is a term used to describe idiopathic, chronic inflammation of the gastrointestinal tract and includes two main phenotypes: Crohn's disease (CD) and ulcerative colitis (UC). Crohn's disease is typified by granulomatous inflammation affecting any part of the gastrointestinal tract but particularly the ileocecal area. Ulcerative colitis is colon-specific and is associated with extensive epithelial damage, crypt abscesses and abundant mucosal neutrophils. Patients with extensive UC or colonic Crohn's disease have an approximately ten-fold increased risk of developing colorectal cancer, which represents the major cause of IBD-associated mortality.

The aetiology of IBD is still not well understood. There is however a growing consensus based both on patient studies and from transgenic animal models of colitis that the intestinal inflammation results from an abnormal response to non-pathogenic intestinal bacteria. Some of the most striking evidence for the involvement of bacteria in IBD pathogenesis comes from studies demonstrating the presence of *E. coli* in ileal mucosal biopsies taken from Crohn's disease patients. These organisms, despite lacking conventional markers of bacterial pathogenicity, are able to invade intestinal cell lines and to live within macrophages without inducing apoptosis. Other studies, which used a broad-specificity 16S ribosomal probe for in situ hybridisation to detect bacteria of all species, found an increase in unidentified bacteria within the mucus layer in both UC and CD.

Given the debilitating nature of IBD, as well as IBD-associated mortality, there is a need to provide new and improved treatments for these conditions. It is therefore an object of the present invention to provide compositions useful in the prevention or treatment of IBD.

According to a first aspect of the present invention, there is provided a composition for use in the prevention or treatment of Inflammatory Bowel Disease

comprising a therapeutically effective amount of a soluble fibre derivable from fruit of the *Musa* spp.

According to a second aspect of the present invention, there is provided the use of soluble fibre derivable from fruit of the *Musa* spp. for the manufacture of a medicament for the prevention or treatment of Inflammatory Bowel Disease.

According to a third aspect of the present invention, there is provided a method for the treatment of Inflammatory Bowel Disease comprising administering to a subject in need of such treatment a therapeutically effective amount of a a soluble fibre derivable from fruit of the *Musa* spp.

By "soluble fibre" we mean fibres capable of being dissolved in an aqueous medium (and also optionally the bloodstream) that comprise soluble oligosaccharides from a non-starch source. Such fibres are capable of passing through the stomach and small intestine to the large intestine without being substantially digested. The fibres may then act as a fermentable substrate for bacteria in the large intestine. Different types of soluble fibre may be derived from common food sources such as fruits, especially apples and oranges; vegetables; oat bran; barley; and legumes. In contrast, insoluble fibres are found in the cell wall of plants and give the plant structure. A common food source of insoluble fibre is wheat bran and fruits with edible skins and seeds, such as strawberries. Insoluble fibres aid digestion. They primarily act as bulking agent (resulting in shorter transit time and increased faecal mass); and also hold on to water as they move through the intestinal tract (softening the stools and thereby helping prevent constipation).

Although we do not wish to be bound by any hypothesis the inventors believe that soluble fibres are useful in the treatment and prevention of IBD based upon their understanding of this scientific field and the work presented in Example 1.

They have recognised that IBD (e.g. ulcerative colitis and Crohn's disease) may represent an altered response to the normal intestinal microbial flora. In particular, they believe there is a possibility that apparently non-pathogenic "harmless" bacteria can cause inflammation if they penetrate the layer of mucus that

coats the intestine and associate themselves closely with the lining cells of the intestine. A critical step in bacterial colonisation of the gut is adherence of the bacterium to the mucosa through fimbrial- and/or surface proteins known as adhesins. These proteins act as lectins recognising glycosyl motifs expressed by host cell-surface glycolipid or glycoprotein and play a key role in bacterial pathogenicity. Aberrant glycosylation of mucosal glycoproteins has been demonstrated in colonic disease, with similar abnormalities having been found in ulcerative colitis, Crohn's disease and colon cancer. The inventors believe that there may be a sub-group of bacteria with adhesins specific to the carbohydrate structures that are expressed in inflammatory bowel disease mucosa. Thus the altered glycosylation of colonic mucosal glycoproteins seen in IBD may represent receptors for specific adhesins of the *E. coli* associated with early inflammatory bowel lesions.

The inventors therefore characterised adherence of inflammatory bowel disease-related bacteria to carbohydrate structures (see Example 1). They found that the adhesion of such bacteria to cell surfaces can be blocked by some, but not all, complex carbohydrates. In particular they found that soluble fibres according to the invention were effective for preventing bacterial adhesion and accordingly have efficacy for preventing or treating IBD. We believe that the soluble fibres may either mimic or compete with bacterial receptors, thus preventing bacterial recruitment and subsequent inflammation.

Soluble fibre according to the present invention may be derived from low molecular weight partially hydrolysed gums (e.g. guar gum, locust bean gum, fenugreek gum or okra gum and other suitable low viscosity edible gums), bovine submaxillary mucin (BSM), alginate or partially hydrolyzed carrageenan. However the inventors have found that soluble fibres derived from the *Musaceae* family (i.e the *Musa* and *Ensete* genera) are particularly useful. Soluble fibres from the *Musa* spp (i.e bananas or plantain) have most efficacy for treating or preventing IBD. 11 *Musa* species are known but the soluble fibres are preferably derived from the cultivars *Musa acuminata*, *Musa balbisiana*, *Musa paradisiaca* or *Musa sapientum*.

A preferred soluble fibre for use in treating or preventing IBD is derived from plantain or green (i.e. unripe) edible bananas.

According to a first method for producing soluble fibre: plantain or banana may be sliced and then boiled in water (preferably sterile water) for between 2 and 60 minutes, preferably about 30 minutes. These sort of boiling times helps to break down starch (the inventors have found that it is undesirable to have a high starch content in the soluble fibres). After boiling the solution should be centrifuged (e.g. at 10,000g for 10 minutes) to separate insoluble material from the supernatant. The pellet should then be discarded and the supernatant may be used as soluble fibre according to the present invention. The fact that the supernatant is useful is particularly surprising in the light of the prior art which suggests that the pulp (i.e the pelleted material) may be medically useful. For instance, Rabbini *et al.* (2001) Gastroenterology 121 p554-560) suggest banana pulp may be used to treat diarrhoea.

Preferred soluble fibre has undergone a treatment with an enzyme capable of hydrolysing starch and thereby removing starch from the soluble fibre. Amylase may be used for such a purpose. Accordingly a preferred protococol for producing the soluble fibre involves the soluble fibre being extracted from boiled plantains and being treated with amylase to remove starch and produce a Non-Starch Polysaccharide (NSP) fraction.

A useful protocol involving the removal of starch and representing a second method for producing soluble fibre, comprises:

(1) Plantain skins are removed from the plantains and the whole fruit (excluding skins) freeze-dried and then milled.

(2) The milled material is then boiled (100°C) for approximately 30mins in water to gelatinise the starch and disperse any starch granules.

(3) The material is then hydrolysed with porcine pancreatic amylase (at 40°C) to ensure all/most starch is removed.

(4) The remaining liquid fraction comprises Non-Starch Polysaccharides (NSP). This is then precipitated and washed with 80% ethanol and centrifuged (at about 10,000g - it will be appreciated that lower centrifugal forces may be employed). The alcoholic supernatant is then discarded and the residue (containing both soluble and insoluble NSP) freeze-dried. This freeze-dried product (which represents soluble fibre according to the invenion) may then be stored and reconstituted when desired.

(5) When soluble fibre is required in solution, the freeze-dried residue is boiled in water for about 30mins; centrifuged at 10,000g for 10 mins and the supernatant used as required.

The supernatant (5) may optionally be freeze dried again to make a preferred (powdered) soluble fibre according to the invention.

The inventors have found that edible matter from plantains (i.e. everything but the skins) constitutes about 50% of the plantain and dry matter constitutes about 31%. The inventors have found that the yield of NSP (following the second method above) is about 3-4% and about 40% of this NSP represents soluble fibre according to the invention.

The inventors have also found that an effective pH for a soluble fibre solution is around pH 7. An increase in pH increases solubility but excessive alkalinity was found to degrade the oligosaccharides in the soluble fibre and result in a decrease in efficacy.

Table 1 illustratres the sugar content of a typical soluble fibre obtained according to the steps (1) - (5) of the second method discussed above. Analysis of the sugar content of a soluble fibre provides a "fingerprint" for soluble fibre from a particular source. It is possible to distinguish between soluble fibre derived from plantain and other plants (e.g. pea-husk or wheat). It will therefore be appreciated that preferred *Musa* derived soluble fibre has a sugar content similar to that given in Table 1.

Table 1

Non-Starch Polysaccharide Constituent Sugars (g/100g dry matter)									Total NSP
Rhamnose	fucose	Arabinose	xylose	mannose	galactose	glucose	Glucuronic acid	Galacturonic acid	g/100g
0.1	0.1	0.3	0.2	0.6	0.3	0.9	0.1	0.8	3.4

Most preferred soluble fibre preparations for use according to the invention are described in the Examples.

IBD may be treated using soluble fibre derived from a natural source (e.g. prepared from Banana or plantain). Alternatively synthetic sugars may be used with the same, or similar composition to the soluble fibre prepared from natural sources.

It will be appreciated that a crude preparation derived from boiling and then centrifuging fruit such as bananas and plantain (i.e the first method discussed above) may be used as soluble fibre according to the invention.

Clinical needs may dictate that such a crude preparation (the supernatant made according to the first method) may need to be used substantially "neat" or even diluted. When this is the case the supernatant (whether diluted or not) may be mixed with a number of other agents that may be added for nutritional reasons, medical reasons or even for the purposes of adjusting the palatability of the soluble fibres for consumption by the subject being treated.

For instance, the soluble fibre may be formulated with a diary product (e.g. milk, a milk shake or yoghurt) or a fruit juice (e.g. orange juice or similar) to produce a palatable drink/beverage with the added benefit that it contains soluble fibre and therefore will be highly suitable as a refreshment for sufferers of IBD.

Alternatively, the crude preparation may be included in a nutritional liquid for enteral feeding. For instance, the supernatant may be mixed with saline or an aqueous solution (other vitamins, minerals and nutrients may be included) for enteral feeding of subjects.

It will be appreciated that concentration of the crude preparation from the first method may be required or alternatively a powder composition is desired. When this is the case the crude extract/supernatant will need to be concentrated/dehydrated.

Freeze-drying or spray drying represent preferred methods for producing a powder comprising soluble fibres according to the invention. Spray drying results in free-flowing granular powder mixes with good flow properties and quick dissolving characteristics.

It will also be appreciated that the freeze-dried powder produced by steps 1 – 4 of the second method discussed above represents powdered soluble fibre according to the invention. A preferred powder is derived from a reconstituted soluble fibre solution produced by steps (1) -(5) of the second method (above) which is subsequently freeze-dried or spray-dried.

Powdered soluble fibre (whether generated according to the first or second methods or otherwise) may be reconstituted as a clear/translucent low viscosity drink/beverage. Reconstitution may be into water or dairy or fruit juices as discussed above. It will be appreciated that the powder may be packaged in a sachet and reconstituted as a drink by a subject when required or desired.

Powder mixes represent preferred embodiments of the invention. Such mixes comprise powdered soluble fibre (as described above) mixed with further ingredients. Such ingredients may be added for nutritional or medical reasons or for improved palatability. The powdered soluble fibre may be mixed with granulated sugars of varying particle sizes to obtain free-flowing powder mixes of varying sweetness.

Alternatively natural sweeteners or artificial sweeteners (e.g. aspartame, saccharin and the like) may be mixed with the powdered soluble fibre for reconstitution as a low calorie/reduced calorie sweetened drink. The powder mix may comprise a mineral supplement. The mineral may be any one of calcium, magnesium, potassium, zinc, sodium, iron, and their various combinations.

It will be appreciated that soluble fibre may advantageously be formulated as a pharamaceutical for use as a medicament (requiring a prescription or otherwise).

Powdered soluble fibre or concentrated liquid soluble fibre may also be incorporated into tablets, lozenges, sweets or other food-stuffs for oral ingestion. It will also be appreciated that such powdered soluble fibre or concentrated liquid soluble fibre may be incorporated into slow-release capsules or devices which may be ingested and are able to release soluble fibre into the intestines over a long period of time.

Soluble fibres may also be microencapsulated. For instance encapsulation may be by calcium-alginate gel capsule formation. Kappa-carrageenan, gellan gum, gelatin and starch may be used as excipients for micro-encapsulation.

Crude preparations, liquid concentrates, powders and the like may be combined with known therapeutic agents for treating IBD. As such the soluble fibre according to the invention may be used in a very effective combination therapy. It will be appreciated that the soluble fibre in solution may act as an ideal vehicle for other therapeutic agents for treating IBD.

The soluble fibre may also be included in combination/synbiotic therapies that include a probiotic portion. The bacteria contained within many probiotic mixtures do not possess adhesive properties so would not be affected by the inclusion of plantain soluble fibre.

The amount of a soluble fibre required by a subject is determined by biological activity and bioavailability which in turn depends on the formulation, mode of administration, the physicochemical properties of the soluble fibres and whether the soluble fibre is being used as a monotherapy or in a combined therapy. Generally, a daily dose for a human adult should be between 0.1g and 100g of freeze-dried or spray-dried powder (however formulated), more preferably the daily dose is between 1g and 30g and most preferably about 15g.

The frequency of administration will also be influenced by the above mentioned factors and particularly the half-life and of the soluble fibres within the subject being treated. For instance, the half-life will be influenced by the health status of the subject, gut motility and other factors.

The soluble fibre is particularly useful when included in pharmaceutical formulation such as a tablet or a capsule. Such formulations may be required to be enterally-coated if bioavailabilty dictates this. Known procedures, such as those conventionally employed by the pharmaceutical industry (e.g. *in vivo* experimentation, clinical trials etc), may be used to establish specific formulations of

pharmaceutical compositions and precise therapeutic regimes (such as daily doses of the compounds and the frequency of administration).

It will be appreciated that conventional "nutriceutical" procedures may be employed to create liquid drinks, powder mixes and food-stuffs comprising the soluble fibre.

Daily doses may be given as a single administration (e.g. a daily tablet for oral consumption or as a single liquid drink). Alternatively the soluble fibre used may require administration twice or more times during a day. As an example, a 100ml orange drink containing 0.1 – 20g of spray dried soluble fibre (preferably 0.3 – 10g of spray dried soluble fibre and more preferably 0.5 –3.0g) may be used to quench thirst at regular intervals throughout the day and thereby deliver a recommended dose.

It will be appreciated that nutritional products supplemented with soluble fibre represent an ideal means for providing subjects with, or at risk of developing, IBD with soluble fibre according to the present invention. Therefore, according to a fourth aspect of the present invention there is provided a nutritional product for use in the prevention or treatment of Inflammatory Bowel Disease comprising a therapeutically effective amount of a soluble fibre derivable from fruit of the *Musa* spp.

The nutritional product may comprise:

(a) a clear, low viscosity, water-like, stable, ready-to-use, bottled, carbonated or non-carbonated drink; or a concentrated clear liquid for reconstitution containing a non-gelling soluble fibre derivable from fruit of the *Musa* spp;

(b) a powder/granular mix to be reconstituted with water or any other orally ingestible liquid as a drinkable liquid, containing a non-gelling soluble fibre derivable from fruit of the *Musa* spp; or

(c) a powder / granular mix mixed into a food stuff (e.g. a chocolate bar, lozenge or the like).

The nutritional product may be as described above and may or may not contain water soluble vitamins, additional mineral supplements, nutritional compounds, antioxidants or flavourings.

The present invention will be further illustrated, by way of examples, with reference to the accompanying drawings in which:

Figure 1 illustrates fimbrial expression by a mucosa-associated E. coli using electron microscopy (some indicated by arrows) in Example 1;

Figure 2 is a graph showing a significant increase in mucosally-associated bacteria in patients with Crohn's disease in Example 1;

Figure 3 shows that significantly more *E. coli* isolated from Crohn's disease tissue possessed heamagglutinating activity as compared to *E. coli* isolated from control patients in Example 1;

Figure 4 shows that agglutinating strains of *E. coli* are able to attach to and invade I-407 cells at a significantly higher level than non-agglutinating strains of *E. coli* (this validates the use of the haemagglutination assay to assess inhibition of bacterial adhesion)

Figure 5 is a graph illustrating that plantain soluble fibre significantly reduced bacterial attachment to cells in Example 1; and

Figure 6 is a graph illustrating that plantain soluble fibre significantly reduced bacterial invasion to cells in Example 1.

EXAMPLE 1.

The inventors conducted studies with the purpose of investigating IBD biopsies for the presence of bacterial populations which were either within the mucus layer; closely associated with the mucosa; or those which had invaded the mucosal layer. Cultured bacteria were fully characterised and those identified as *E. coli* were studied for their ability to adhere to human red blood cells. Adherent *E. coli* were further characterised for the presence of pathogenicity genes and known adhesins, their binding specificities and their ability to invade human intestinal cell lines.

1.1 MATERIALS AND METHODS

1.1.1 Patients

60 patients were studied. 18 with ulcerative colitis, 14 with Crohn's disease and 28 normal controls. Biopsies were taken during colonoscopy or sigmoidoscopy for the majority of patients, one set of biopsies was taken from a resection sample from a normal patient with chronic inertia.

1.1.2 Bacterial Culture

Four biopsies were taken either from resected tissue samples (one patient) or at colonoscopy (59 patients). Biopsies were placed in 10mls of physiological saline and transported on ice to the laboratory. The biopsies were placed in a 1.5ml eppendorf containing 500µl of 0.016% dithiothreitol (DTT) solution for 15 minutes to remove the mucus layer. 50µl of DTT supernatant was plated onto MacConkey agar and incubated at 37°C for 24hrs. The biopsies were then separated into two 1.5ml eppendorfs containing either 500µl sterile saline or 500µl gentamicin (50ml/L). After 30 mins the biopsies were washed 4 times by transferring them into consecutive 1.5ml eppendorfs containing 500µl saline. After the fourth wash, one biopsy from each eppendorf (i.e one of the gentamicin-treated biopsies and one of the non-gentamicin-treated biopsies) was placed into sterile distilled and de-ionised water, the other biopsy from each eppendorf was placed in 500µl saline. After a further 30 mins, 50µl solution from each of the final eppendorfs were plated onto MacConkey agar plates. After 24hrs incubation at 37°C, colony counts were performed.

1.1.3 Bacterial Identification

After the colony counts had been performed the bacterial colonies were characterised and 5 of each colony type were subcultured onto nutrient agar. Gram staining was performed and any bacteria identified as Gram negative rods were further identified using API20E bacterial identification kits (BioMerieux, Fr). Bacterial samples from each subcultured colony were stored on Protect beads at –80°C.

1.1.4 Antibiotic Resistance Assay

A single well isolated bacterial colony was suspended in 5mls of sterile saline using colonies of bacteria taken from a nutrient agar plate. 100μl of the bacterial suspension was plated onto sensitivity agar plates. Antibiotic discs were then dispensed onto the plate. The following antibiotics were used: Gentamicin, Trimethoprim, Sulphonomide, Ampicillin, Penicillin, Tetracycline, Naladixic acid, Chloramphenicol.. The bacterial plates were incubated at 37°C overnight. The diameters of bacterial growth around the discs were measured and the degree of bacterial resistance to the antibiotics was calculated.

1.1.5 Bacterial growth conditions for maximum fimbrial expression

Maximum fimbrial expression was seen after bacterial colonies grown overnight on nutrient agar, suspended in sterile saline, and then left at room temperature for 48hrs. Fimbrial expression was confirmed using EM and may be seem in Figure 1.

1.1.6 Haemagglutination Assays

Blood was taken from a healthy human volunteer and placed into a heparinised container. The blood was washed three times in PBS by centrifugation at 1000rpm for 5 minutes and by resuspending the pellet in fresh PBS. After the third wash, the pellet was resuspended in PBS so as to give a 1% red blood cell solution. Bacteria identified as *E. coli*, and 5 strains of CD-ileal associated *E. coli* were grown so as to obtain maximum fimbrial expression (see above). Bacteria were suspended in saline to give an OD equal to MacFarland standard 5. Doubling dilutions of bacterial suspension were performed in 96 well U-bottomed haemagglutination plates (Dynex technologies) to a final volume of 100μl per well. 50μl of 1% rbcs was added to each

well. The plate was tapped gently to mix the contents of the wells and was left at room temperature for approximately 2hrs.

1.1.7 Haemagglutination assays using the human rbc panel

All *E. coli* were tested against a panel of rbcs of known antigenic type. These included ID panel 1 (26 antigens including Lewis, Lutheran, M, N, S, P, K, Kidd, Rhesus and others), A1rr cells, Brr cells (ABO blood groups) (NBS reagents, Liverpool, UK) and adult ii cells (Diagnostics Scotland, Edinburgh, UK).

1.1.8 Haemagglutination assays with different oligosaccharides/fibres

Further characterisation of bacterial haemagglutination was carried out using a panel of oligosaccharides/fibres. The structures tested were: mannose (0.4M), fucose (0.4M), galactose (0.4M), fetuin (50μg/ml), asialofetuin (50μg/ml), bovine submaxillary mucin (BSM), hydrolysed BSM, ovine submaxilliary mucin (OSM), lactose, lactulose, siayl Tn, GalGalNAc, N-Glycolyneuraminic acid, N-acetylneuraminic acid, 3'-n-acetylneuraminyl-lactose, 6'-n-acetylneuraminyl-lactose, melibiose, plantain, stachyose, milk derived galacto-oligosaccharide, raffinose, pectin, polygalacturonic acid, and paralacto-n-hexaose.

1.1.9 PCR

Bacteria identified as agglutinating *E. coli* were screened both for pathogenicity genes associated with virulent strains of *E. coli* and for known adhesin genes. These were: VT1,VT2, VT2e, STI, STII, LTI, Einv, Eagg, CNF1, CNF11, EaeA, EaeB, EAF, Bfp, CDT, EAST, Hly, HlyA, pap, sfa, afa, M-agglutinin, fimH.

1.1.10 Pulse field gel electrophoresis

Agglutinating strains of *E. coli* were tested for clonality using Pulse field gel electrophoresis.

1.1.11 Attachment and invasion assays

Agglutinating bacteria were tested for their ability to adhere to and invade the intestinal cell lines I-407 and HT-29. *Shigella sonni* was used as a positive control for invasion and *E. coli* K12, an adherent but non-invasive *E. coli*, was used as a negative

control for invasion. Cells were maintained in DMEM (Dulbecco's modified eagles medium – Sigma, UK) at 37°C, 5% CO_2, supplemented with 10% (vol/vol) heat inactivated fetal calf serum (Sigma, UK), 1% L-glutamine, penicillin and streptomycin. Monolayers were seeded into 24-well tissue culture plates (Costar, UK), with $4x10^5$ cells/well. Cells were incubated for 20hrs. The monolayers were washed twice with phosphate buffered saline (PBS). Bacteria were grown so as to maximise fimbrial expression (48hrs in saline). Each monolayer was infected with approximately $7x10^6$ bacteria/well in culture medium without antibiotic. After 3hrs incubation at 37°C, the monolayers were washed three times with PBS. To determine bacterial invasion, wells were treated with fresh culture medium containing gentamicin (100μg/ml), the purpose of this is to kill any extracellular bacteria as gentamicin cannot penetrate human cells. After one hour at 37°C, the monolayers were washed three times in sterile PBS. Monolayers were lysed by adding deionised water containing 1% Triton X-100 for 5 minutes. Ten-fold dilutions of the cell lysate were performed and 50μl was plated onto nutrient agar plates. Plates were incubated at 37°C and colony forming units (CFUs) were counted after 24hrs. Bacterial attachment was calculated as total CFU counts multiplied by the dilution factor, this has also been expressed as a percentage of the total number of bacteria used to inoculate the wells. Bacterial invasion was defined as the percentage of the attached bacteria which survived gentamicin treatment. A time course of bacterial attachment and invasion was determined using only I-407 cells over a period of 5hrs.

1.1.12 Effect of plantain soluble fibre on attachment and invasion of bacteria

The effect of pre-incubating bacteria with plantain on attachment and invasion of *E. coli* was studied.

1.1.12.1 Preparation of freeze-dried plantain soluble fibre: Skins were removed from plantains and the whole fruit (excluding skins) freeze-dried and then milled. It was then boiled (100°C) for approximately 30mins in water to gelatinise the starch and disperse any starch granules. It was then hydrolysed with porcine pancreatic amylase (at 40°C) to ensure all the starch was removed. The Non-Starch Polysaccharides (NSP) fraction was precipitated and washed with 80% ethanol and centrifuged (about 10,000g). The alcoholic supernatant was then discarded and the

residue (containing both soluble and insoluble NSP) was freeze-dried. This freeze-dried sample represents a powdered soluble fibre according to the invention.

1.1.12.2 Preparation of plantain soluble fibre solution: When a sample of soluble fibre was required the freeze-dried residue (1.1.12.1) was boiled in water for 30mins, centrifuged at 10,000g for 10 mins and the supernatant taken as the soluble fibre of plantain.

1.1.12.3 Preparation of refined freeze-dried plantain soluble fibre: the supernatant (1.1.12.2) was freeze-dried (as described above) to form a preferred powdered soluble fibre according to the invention

1.1.13 Preparation of bacteria for use in attachment and invasion assays.

1ml of the bacterial cultures, which had been grown in sterile saline to achieve maximum fimbrial expression, were pipetted into two eppendorfs. The tubes were centrifuged at 10 000g for 5 minutes. The supernatant was discarded and the pellet from one tube was resuspended in 1ml of soluble plantain solution. The other pellet was resuspended in 1ml sterile saline. Both eppendorfs were rotated in the cell-spinner for 30mins. The bacterial suspensions were centrifuged as before and the pellets were washed twice in sterile saline. The pellets were finally resuspended in 1ml of sterile saline and were used in the attachment and invasion assay as before.

1.2 RESULTS

1.2.1 Increased mucosally-associated bacteria in Crohn's disease

Bacteria were identified using the microbiological techniques described in the Methods section.

In total, of 707 bacterial colonies sub-cultured, 406 were Gram negative and 292 of these were identified as *E. coli*. Sensitivity to antibiotics such as gentamicin, trimethoprim, sulphonomide, ampicillin, penicillin, tetracycline, naladixic acid and chloramphenicol was examined to further characterise the bacteria (data not shown).

Mucus-associated bacteria were cultured from the supernatent of dithiothreitol (DTT)-treated biopsies (DTT removes the mucus layer from the biopsy exposing the mucosa). No significant difference in numbers of mucus-associated bacteria was found between patient groups (data not shown). However figure 2 shows a significant increase in mucosally-associated bacteria in patients with Crohn's disease compared to control patients with 79% (11/14) of CD patients being positive for mucosally-associated bacteria compared to 39% (11/28) of control patients (*P*=0.038; Chi-square). Mucosally-associated bacteria are those that can be recovered from the mucosal surface after surface mucus has been removed by DTT treatment and extensive washing.

This data indicate, as suspected by the inventors, that there is an increase in mucosally-associated bacteria in IBD.

1.2.2 Mucosally-associated *E. coli* express haemagglutinins

Figure 3 illustrates that significantly more *E. coli* isolated from Crohn's disease tissue possessed haemagglutinating activity as compared to *E. coli* isolated from control patients. Haemagglutinating *E. coli* were identified in 39% (5/14) of CD patients compared to 4% (1/28) of controls (P=0.01). An increased number of bacteria from UC patients was also able to agglutinate.

It was also found that bacteria capable of agglutinating red blood corpuscles (rbcs) from a healthy human volunteer, were also able to agglutinate rbcs from a panel of rbcs of known antigenic type supplied by the blood transfusion centre. Crohn's disease-associated strains of *E. coli* were also shown to have selective binding to the rbc panel. The binding pattern of this *E. coli* suggested that it possessed a blood group 'M'- specific adhesin. This bacterium was further characterised using PCR.

These data suggest that mucosally associated *E coli* interact with intestinal cells by means of interaction with glycosylated proteins on the cell surface. This further confirmed the inventors hypothesis relating to the mechanism by which bacteria invade the intestine in IBD.

1.2.3. Mucosally-associated E coli adhere to and invade intestinal cells in culture

16 different strains of agglutinating *E. coli* were characterised according to methods 1.1.4, 1.1.9 and 1.1.10 and subjected to attachment and invasion assays according to method 1.1.11. The human colonic cell-line, HT29 and the human intestinal cell line, I-407 were used in the experiments.

All agglutinating *E. coli* were shown to adhere to both HT29 and I407 cell-lines, but invasion in the main, was seen only in I407 cells (see Table 2). Invasion and adherence were calculated by using the mean number of colony forming units (CFU's) recovered from non-gentamicin and gentamicin-treated cells (n=4). (Gentamicin kills all non-invading E coli but cannot penetrate the intestinal cells so leaves bacteria that have invaded intact). LF82 is an adherent and invasive *E. coli* (AIEC) isolated from an ileal Crohn's disease lesion. *Shigella sonnei* was used as a positive control for invasion, and *E. coli* K12 was used as a negative control for invasion. It is known to adhere to cells via a mannose-binding adhesin but is non-invasive.

Further experiments were performed to compare agglutinating *E. coli* (such as those from subjects with IBD) with non-agglutinating *E. coli*. in respect of their ability to attach and invade I-407 cells. Figure 4 illustrates a correlation between the ability of a bacterium to agglutinate and adhere to intestinal cells. This therefore further suggests that bacteria in IBD invade the mucosa by a targeting mechanism involving glycoproteins.

TABLE 2: Invasion and adherence of agglutinating bacteria

Haemagglutinating *E. coli strain* (except *)	Original inoculum (bacteria x10^6/well)	N° BACTERIA ATTACHED (x10^4/well)		% INVASION	
		I-407 CELLS	HT-29 CELLS	I-407 CELLS	HT-29 CELLS
1	2.20	1.27 ± 0.83	0.05 ± 0.1	14.62 ± 18.89	0.00
2	10.40	5.56 ± 3.25	0.33 ± 0.19	0.99 ± 2.99	0.00
3	7.60	7.23 ± 3.99	0.025 ± 0.05	4.23 ± 6.54	0.00
4	22.00	2.49 ± 1.32	0.58 ± 0.27	1.00 ± 2.87	0.00
5	3.80	35.4 ± 14.14	28.00 ± 0.57	0.23 ± 0.74	0.00
6	7.00	0.34 ± 0.36	0.16 ± 0.19	0.00	0.00
7	1.20	1.18 ± 11.79	0.01 ± 0.02	2.98 ± 3.50	0.00
8	1.80	29.70 ± 11.74	3.00 ± 1.95	0.03 ± 0.10	0.17 ± 0.51
9	3.60	78.40 ± 4.53	4.73 ± 0.71	0.31 ± 1.49	0.11 ± 1.42
10	8.80	4.82 ± 1.62	0.31 ± 0.25	1.77 ± 5.75	0.00
11	15.00	2.51 ± 1.32	0.03 ± 0.04	6.79 ± 9.05	0.00
12	2.40	11.30 ± 2.69	0.31 ± 0.23	9.87 ± 21.71	0.00
13	6.60	27.60 ± 10.75	3.54 ± 0.70	0.65 ± 1.50	1.98 ± 13.57
14	21.80	15.00 ± 3.11	0.25 ± 0.27	10.9 ± 6.61	0.00
15	2.20	0.29 ± 0.22	0.44 ± 0.57	0.00	0.00
16	4.80	3.04 ± 1.82	0.47 ± 0.28	2.30 ± 4.96	0.00
LF10	9.00	56.30 ± 3.25	61.20 ± 12.16	0.82 ± 7.22	0.00
LF82	11.60	2.06 ± 0.94	0.45 ± 0.33	12.90 ± 38.99	12.36 ± 29.87
K12	9.40	9.80 ± 3.11	0.40 ± 0.25	0.00	0.00
**Shigella sonnei*	4.00	2.34 ± 1.91	3.64 ± 1.13	40.38 ± 30.43	3.99 ± 15.57

1.2.4 The effect of soluble fibre on bacteria isolated from subjects with IBD

Soluble fibre (prepared according to 1.1.12.2) and other carbohydrates were tested for their ability to modulate agglutination, attachment and invasion of cells from subjects with IBD.

1.2.4.1 Agglutination: In all cases agglutination was inhibited by soluble plantain fibre and bovine submaxillary mucin (BSM) but not following mild acid hydrolysis of BSM to remove sialic acid and fucose.

A range of other carbohydrates and glycoconjugates, including mannose, fucose, galactose, lactose, lactulose, siaylosyl-Tn, Galβ1-3GalNAc, N-

glycolyneuraminic acid, N-acetylneuraminic acid, 3'-n-acetylneuraminyl-lactose, 6'-n-acetylneuraminyl-lactose, melibiose, stachyose, milk derived galacto-oligosaccharide, raffinose, polygalacturonic acid, and paralacto-n-hexaose, fetuin, asialofetuin, ovine submaxilliary mucin and pectin were non-inhibitory.

1.2.4.2 Inhibition of bacterial attachment by plantain soluble fibre: Bacterial strains 9, 12 and 14 (as identified in Table 2) were Figure 4 illustrates that plantain soluble fibre significantly reduced bacterial attachment of bacterial strains 9 and 12 to I-407 cells. No significant difference can be seen for strain 14 although limited attached was seen for this strain in the presence or absence of soluble fibre.

1.2.4.3 Inhibition of bacterial invasion by plantain: Figure 5 illustrates that bacterial invasion is reduced by pre-incubation of each of three bacterial strains (strains 9, 12 and 14 from Table 2) with plantain soluble fibre.

1.3 DISCUSSION

These data demonstrate that IBD such as Crohn's disease is associated with an increased prevalence of mucosally-associated *E. coli*. These *E. coli* lacked known pathogenicity genes but include a high proportion which express haemagglutinins which allow them to adhere to, and invade, intestinal epithelial cell lines. The inventors believe that the adhesive and invasive nature of the bacterium cause an inflammatory condition and thereby characterise IBD.

The inventors established a link between IBD and the ability of bacteria from subjects with IBD to agglutinate. They therefore reasoned that binding of bacteria in IBD must be regulated by carbohydrates and tested many oligosaccharides to evaluate whether or not they would inhibit the negative effects of *E. coli* in IBD. Most of the sugars/oliogosaccharides tested failed to modulate the bacteria. However, surprisingly, soluble fibre from plantain inhibited *E. coli*. This led the inventors to realise that soluble fibre according to the invention comprises bacteria modifying oligosaccharides for therapeutic use in IBD such as Crohn's disease.

It will be appreciated that these data suggest that soluble fibre according to the invention will also be useful for treating or preventing the development of other

gastrointestinal conditions which may be characterised by bacterial adhesion and invasion (e.g. colon cancer, diarrhoeal disease/infections, gastroenteritis or Type 1 Glycogen-storage disease.

<u>EXAMPLE 2:</u> Production of a powder of Plantain Soluble Fibre for use according to the Invention.

250g of chopped plantain pulp was dropped into 1,000ml of boiling and then treated as described in 1.1.12.1 The freeze-dried yield of Soluble Fibre (1.1.12.2) was approximately 4.0g

<u>EXAMPLE 3:</u> Production of a Powder mix of Plantain Soluble Fibre for use according to the Invention

3.0g of freeze dried soluble fibre powder (Example 2) was mixed with 0.5g powdered citric acid, 26.3g of granulated sugar and 0.2g of a standard spray-dried mix of flavouring.

This mixture represents a free-flowing powder formulation (containing 3.0g of soluble fibre) that is suitable for packaging in a sachet. The powder mix may be diluted to taste and drunk when required by a subject suffering from IBD

<u>EXAMPLE 4:</u> Production of an Orange Drink for use according to the Invention.

(a) 100ml of crude preparation (prepared according to method 1.1.12.2) was mixed with 100mls of double concentrate orange juice (orange juice concentrate diluted in water to double strength).

(b) 3.5g of freeze-dried powder (prepared according to the method 1.1.12.1) was dissolved in 100mls of orange juice (or alternatively with orange juice concentrate and water).

(c) 2.5g of freeze-dried powder (prepared according to the method 1.1.12.3) was dissolved in 100mls of orange juice (or alternatively with orange juice concentrate and water).

The orange drink preparations (a, b or c) may be consumed by a subject immediately, refrigerated for later consumption or sealed in a bottle or carton for a longer shelf life.

It will be appreciated that orange juice may be readily substituted with a palatable alternative.

CLAIMS

1. A composition for use in the prevention or treatment of Inflammatory Bowel Disease comprising a therapeutically effective amount of a soluble fibre derivable from fruit of the *Musa* spp.

2. The composition according to claim 1 wherein the soluble fibre is derived from boiling plantain or green bananas.

3 The composition according to claim 1 or 2 wherein the soluble fibre is treated to remove starch

4 The composition according to any preceding claim wherein the soluble fibre is obtained by:

(a) freeze-drying and milling skinned whole fruit;
(b) boiling the milled material to gelatinise any starch and dispere any starch granules;
(c) hydrolysing starch in the material with amylase;
(d) precipitating and washing the material with ethanol and freeze drying to produce a residue; and
(e) reconstituting the freeze-dried residue, boiling and centrifuging; wherein the supernatant from the centrifugation comprises the soluble fibre.

5. The composition according to claim 1 in powder form.

6. The composition according to claim 5 wherein the powder comprises the freeze-dried residue of claim 4(d) or the supernatant of claim 4(e) which is spray-dried or freeze-dried.

7. The composition according to any preceding claim further comprising at least one of flavouring, sugars, sweetener, anti-oxidants, minerals or vitamins.

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8. The composition according to any preceding claim for the prevention or treatment of Crohn's Disease.

9. A use of soluble fibre derivable from fruit of the *Musa* spp. for the manufacture of a medicament for the prevention or treatment of Inflammatory Bowel Disease.

10. The use according to claim 9 wherein the medicament is a composition according to any one of claims 1 - 8 in combination with a pharmaceutically acceptable carrier.

11. The use according to claim 10 wherein the medicament is a liquid, capsule or tablet for oral consumption

12. A method for the treatment of Inflammatory Bowel Disease comprising administering to a subject in need of such treatment a therapeutically effective amount of a soluble fibre derivable from fruit of the *Musa* spp.

13. A nutritional product for use in the prevention or treatment of Inflammatory Bowel Disease comprising a therapeutically effective amount of a soluble fibre derivable from fruit of the *Musa* spp.

14. A nutritional product according to claim 13 in the form of a beverage or drink.

15. The nutritional product according to claim 14 comprising between 1g/100ml and 30g/100ml of soluble fibre.

16. A nutritional product according to claim 13 in the form of a powder or powder mix.

17. A nutritional product according to claim 13 in the form of a food bar or other solid food stuff.

FIG. 1

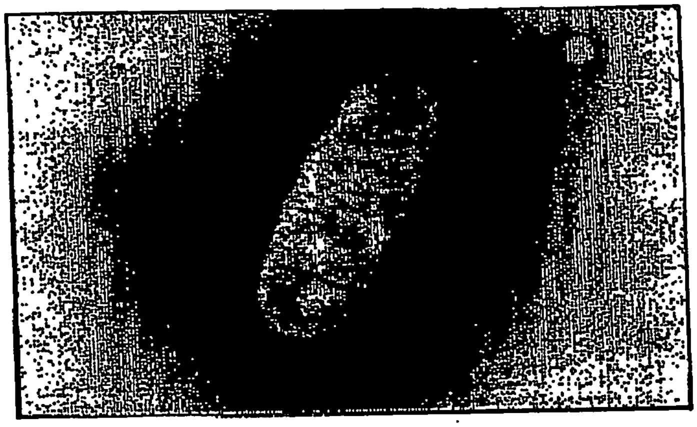


FIG. 2

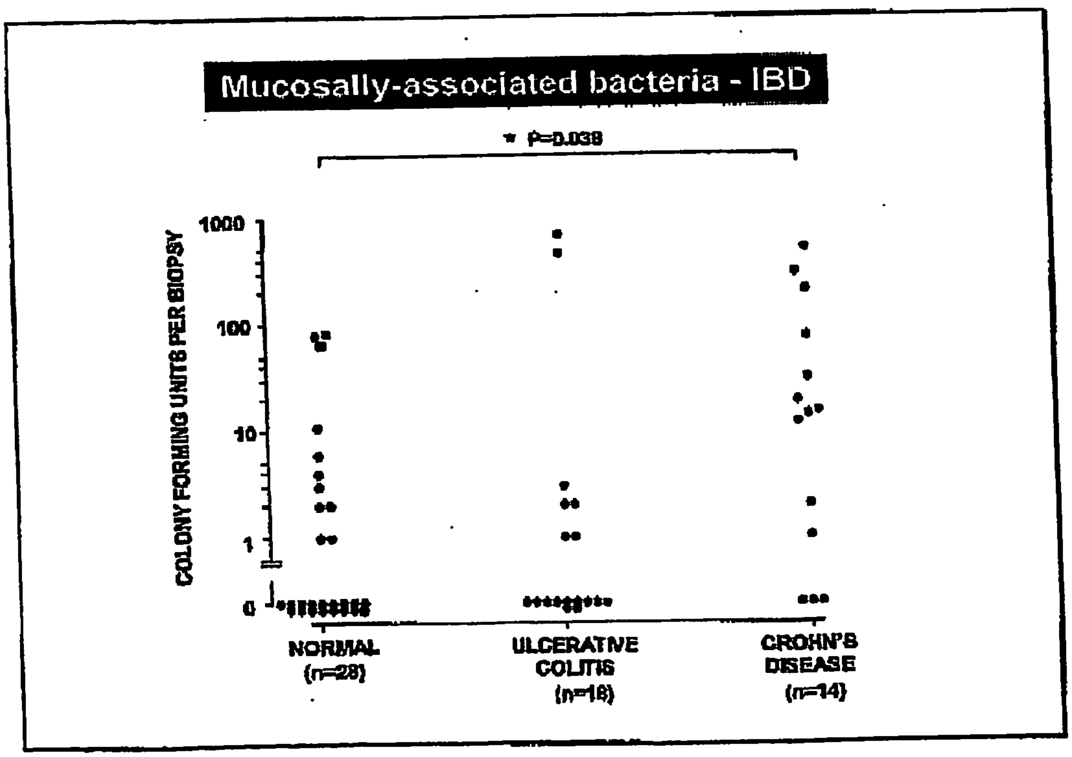

Mucosally-associated bacteria - IBD
* P=0.038
COLONY FORMING UNITS PER BIOPSY
1000
100
10
1
0
NORMAL (n=28)
ULCERATIVE COLITIS (n=18)
CROHN'S DISEASE (n=14)

FIG. 3

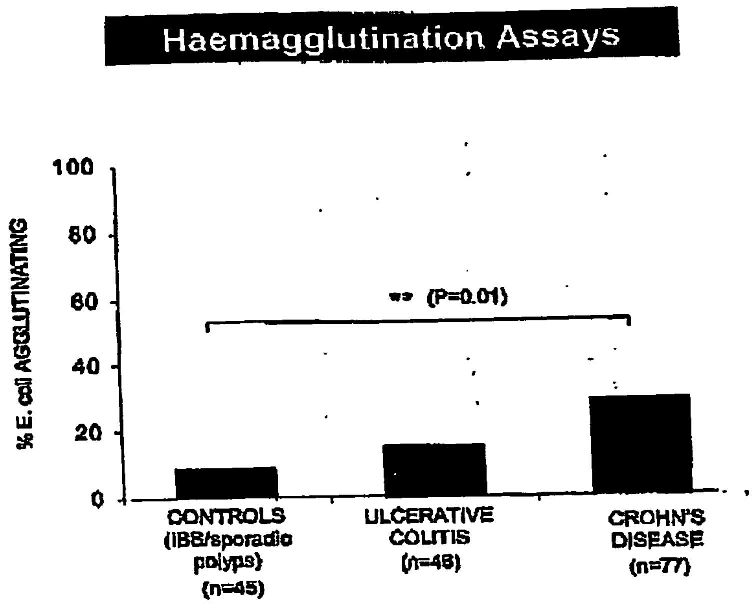

Haemagglutination Assays
100
80
60
40
20
0
% E. coli AGGLUTINATING
** (P=0.01)
CONTROLS (IBS/sporadic polyps) (n=45)
ULCERATIVE COLITIS (n=48)
CROHN'S DISEASE (n=77)

FIG. 4

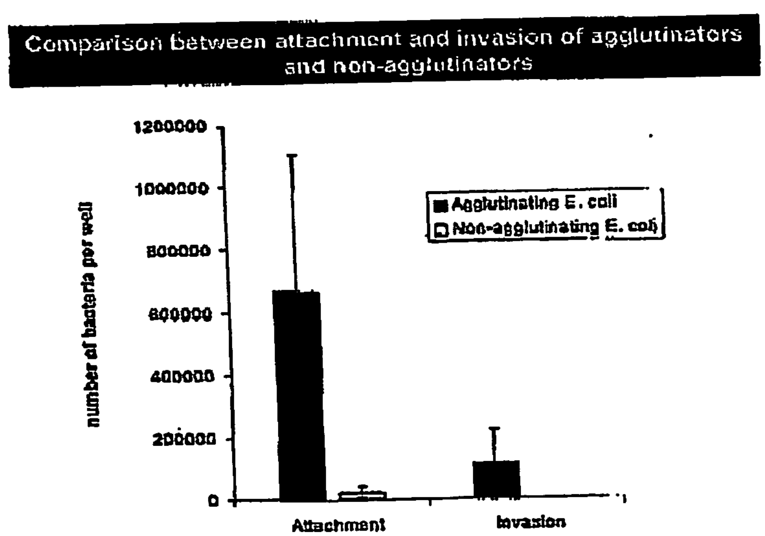

Comparison between attachment and invasion of agglutinators and non-agglutinators
1200000
1000000
800000
600000
400000
200000
0
number of bacteria per well
Agglutinating E. coli
Non-agglutinating E. coli
Attachment
Invasion

FIG. 5

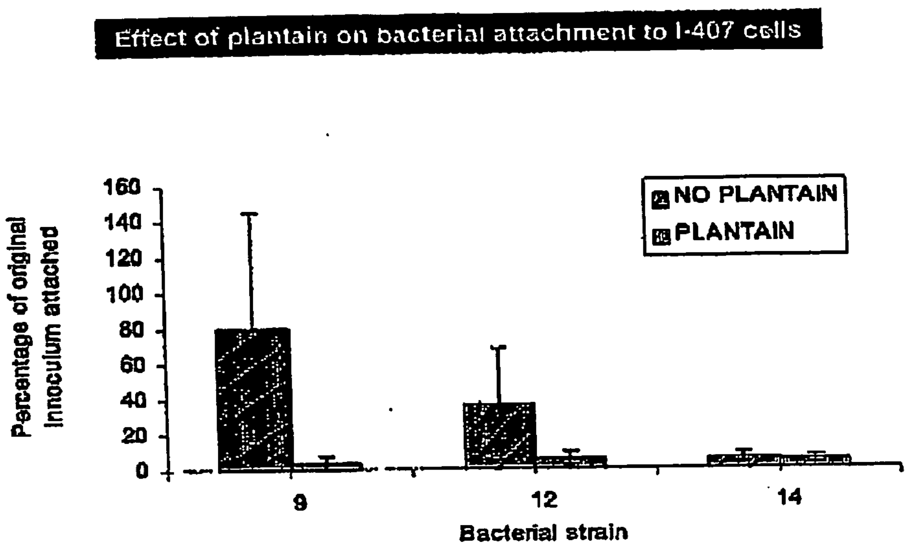
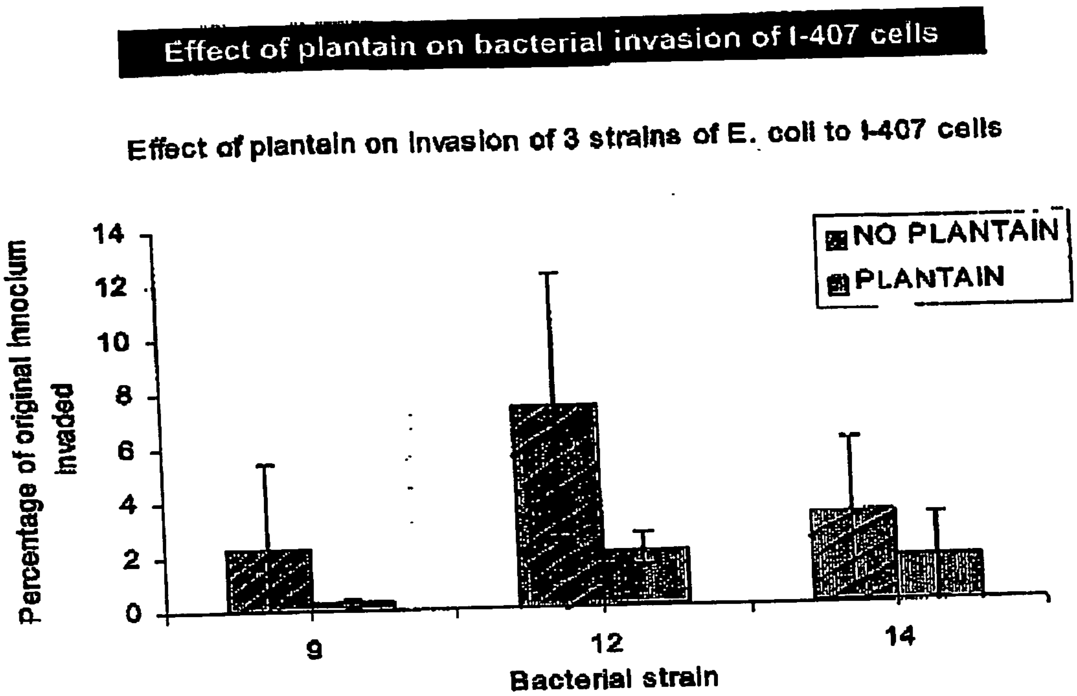

Effect of plantain on bacterial attachment to I-407 cells
Percentage of original Innoculum attached
160
140
120
100
80
60
40
20
0
NO PLANTAIN
PLANTAIN
9
12
14
Bacterial strain

FIG. 6


Effect of plantain on bacterial invasion of I-407 cells
Effect of plantain on invasion of 3 strains of E. coli to I-407 cells
NO PLANTAIN
PLANTAIN
Percentage of original innoclum invaded
14
12
10
8
6
4
2
0
9
12
14
Bacterial strain

PCT Application
PCT/GB2004/000521